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Evaluation of Soybean Meal as a Replacement for Marine Animal Protein in Diets for Shrimp (*Penaeus vannamei*)

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ABSTRACT

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Six isonitrogenous, isocaloric diets containing 0, 14, 28, 42, 56 and 70% soybean meal as replacement of 0, 20, 40, 60, 80 and 100% of animal protein, which consisted of 53% anchovy fish meal, 32% shrimp head meal and 15% squid meal, were fed to juvenile *P. vannamei* to satiation six times per day for 56 days. Shrimp fed the three lowest dietary levels of soybean meal (0, 14 and 28%) had similar weight gains of 6.77 ± 0.36, 6.91 ± 0.40 and 6.56 ± 0.23 g, respectively. Weight gains declined significantly ($P < 0.05$) to 6.15 ± 0.24, 5.12 ± 0.14 and 2.34 ± 0.10 g as the dietary soybean levels increased to 42, 56 and 70%, respectively. Protein and fat gains followed the pattern of weight gains. Survival rates obtained for various treatments ranged from 86.7 to 98.3%. There were significant differences ($P < 0.05$) among the survival rates. However, these differences could not be attributed to the dietary levels of soybean meal. Dry matter feed intakes were not significantly different ($P > 0.05$) for diets containing 0, 14, 28 and 42% soybean meal (10.94 ± 0.43, 11.02 ± 1.02, 10.22 ± 0.35 and 9.79 ± 0.62 g, respectively). Feed intakes decreased ($P < 0.05$) to 8.43 ± 0.34 and 6.15 ± 0.45 g for the 56 and 70% soybean diets. Pellet water stability was inversely related to level of dietary soybean meal. Feed conversion, protein efficiency ratio and apparent protein utilization were similar for diets having 0 to 56% soybean meal. The moisture content was highest ($P < 0.05$) for shrimp fed the 70% soybean diet; moisture content of shrimp fed other diets was essentially the same. There were no significant differences ($P > 0.05$) in whole body composition of lipid, ash, calcium or potassium of shrimp on the various diets. Body phosphorus percentage decreased significantly ($P < 0.05$) when soybean meal levels exceeded 42%. The 70% soybean meal diet was utilized very poorly by the shrimp.

INTRODUCTION

Commercial shrimp feeds usually contain 30 to 50% crude protein, composed mostly of marine animal protein products such as fish, shrimp and squid meals.

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These feed materials have high nutritive value and palatability but are expensive and not readily available (Lim and Persyn, 1989). Soybean meal, the most commonly used plant protein in feeds for domestic animals and warmwater fish, is available worldwide at a lower cost than marine animal proteins, but its utilization in shrimp feeds is still limited.

Several studies have evaluated soybean meal as a partial or complete replacement of fish meal, shrimp meal, squid meal or a combination of fish and shrimp meals for various species of shrimp (Forster and Beard, 1973; Sick and Andrews, 1973; Hirata et al., 1975; Balaz and Ross, 1976; Fenucci and Zein-Eldin, 1976; Colvin and Brand, 1977; Fenucci et al., 1980; Lawrence et al., 1986; Akiyama, 1989). Many of the results reported are contradictory. This may reflect differences in species and size of shrimp, composition and nutrient content of the basal diets, quality of the test ingredients, feeding management and culture conditions. Generally, however, high levels of dietary soybean meal or complete substitution of animal proteins resulted in poor growth and feed efficiency.

No studies have yet examined soybean meal as a substitute for a mixture of fish, shrimp and squid meals. Therefore, the objective of the present study was to compare the growth, feed conversion, survival and body composition of juvenile *Penaeus vannamei* fed diets containing various levels of soybean meal as substitutes for a marine animal protein mix. Feed consumption, protein efficiency ratio, apparent protein utilization and pellet water stability were also determined for the various diets.

MATERIALS AND METHODS

Six isonitrogenous and isocaloric practical diets were formulated to contain 0, 14, 28, 42, 56 and 70% commercial solvent-extracted soybean meal as replacements of 0, 20, 40, 60, 80 and 100%, respectively, of a marine protein mix, consisting of 53% anchovy fish meal, 32% shrimp head meal and 15% squid meal (Table 1). All diets were calculated to contain approximately 35% crude protein and 3450 kcal/kg metabolizable energy (ME). Since the energy values of various feedstuffs are not available for shrimp, the ME values were calculated on physiological fuel values of 4 kcal/g for protein and carbohydrate and 9 kcal/g for lipid (Maynard and Loosli, 1979). The diets were maintained isocaloric by adjusting the level of cod-liver oil and corn starch. Cholesterol, calcium, total phosphorus and potassium levels were constant in all diets. Diatomaceous earth was used as a filler and kelgin and sodium hexametaphosphate as a binder. Alpha cellulose was used to adjust the dietary level of crude fiber.

All feed ingredients were ground and passed through a 0.5-mm mesh sieve. The dry ingredients of each diet were mixed thoroughly in a Hobart mixer before the oil was added. After the oil was dispersed, approximately 360 ml of

TABLE 1

Percentage composition and proximate analysis of experimental diets

Ingredients and nutrient content	Diet number					
	1	2	3	4	5	6
Ingredients						
Animal protein mix[1]	53.30	42.70	32.00	21.30	10.70	-
Soybean meal	-	14.00	28.00	42.00	56.00	70.00
High gluten wheat flour	10.00	10.00	10.00	10.00	10.00	10.00
Corn starch	21.10	16.90	12.75	8.45	4.30	-
Cod-liver oil	2.10	2.45	2.85	3.20	3.50	3.95
Cholesterol	0.50	0.55	0.60	0.65	0.70	0.75
Soybean lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Binder[2]	3.50	3.50	3.50	3.50	3.50	3.50
Calcium carbonate	-	0.70	1.30	2.00	2.65	3.30
Dicalcium phosphate	-	0.80	1.80	2.70	3.60	4.50
Dipotassium phosphate	1.90	1.50	1.10	0.70	0.35	-
Mineral mix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin mix[4]	2.00	2.00	2.00	2.00	2.00	2.00
Alpha cellulose	0.70	0.55	0.40	0.30	0.15	-
Diatomaceous earth	2.90	2.35	1.70	1.20	0.55	-
Nutrient content (percent on as fed basis)						
Moisture	9.30	9.80	9.70	9.00	9.00	7.20
Crude protein	36.91	36.53	37.47	35.40	36.22	37.62
Crude fat	7.53	7.31	6.86	6.73	6.20	6.45
Ash	14.87	14.34	13.91	13.74	13.01	13.09
Calcium	1.90	1.98	2.08	2.37	2.18	2.40
Phosphorus	1.81	1.87	1.94	1.93	1.97	2.10
Potassium	1.27	1.26	1.44	1.27	1.46	1.57
Percent animal protein substituted by soybean meal	0	20	40	60	80	100

[1]Mixture consisting of 53% anchovy fish meal, 32% shrimp meal and 15% squid meal.
[2]Mixture provides 2.5% kelgin and 1.0% sodium hexametaphosphate.
[3]Modified BT_m salt mixture plus the following minerals (mg/kg diet): zinc, 31.5; iodine, 4.8; selenium, 0.4; cobalt, 0.4.
[4]Vitamin mixture providing the following vitamins (mg/kg diet): vitamin A, 80 000 IU; vitamin D_3, 2000 IU; vitamin E, 500 IU; vitamin K, 20; niacin, 300; riboflavin, 60; pyridoxine, 57; thiamin, 60; pantothenic acid, 147; biotin, 2; folic acid, 20; vitamin B_{12}, 0.1; choline chloride, 3000; inositol, 200; vitamin C, 500.

tap water per kg of dry diet was added. The resulting dough-like mixture was then extruded through a Hobart meat grinder into 3-mm diameter pellets. The pellets were dried in a forced-air Despatch oven at 100°C for 8 min. After rapid cooling, the pellets were packed in sealed plastic bags and stored in a freezer at $-30^\circ C$ until used. Prior to feeding, all diets were broken into small pieces of 3 to 5 mm length.

Juvenile *P. vannamei* were obtained from a commercial shrimp farm (Amorient Aquafarm Inc., Kahuku, Hawaii) and acclimated for 13 days. During this period, they were fed a commercial feed twice daily. After acclimatization, shrimp of 0.7 to 1.5 g size (average 1.02 ± 0.06 g) were selected, individually weighed and stocked into 24 flow-through (0.85 l/min) 65-l glass aquaria at a stocking density of 15 shrimp each. Four aquaria were randomly assigned to each of the experimental diets. Each aquarium, filled with 52 l of water, was provided with a tight-fitting lid, plastic netting shelter and continuous aeration through an airstone. Any shrimp which died within 72 h after stocking was replaced by a shrimp of similar size.

Each test diet was fed to shrimp six times daily to satiation for 56 days. Feeds were offered three times in the morning between 08.00 and 10.00 h and three times in the afternoon between 14.00 and 16.00 h. The quantity of feed consumed per aquarium was determined daily.

All aquaria were cleaned daily by siphoning off accumulated waste materials and exuviae. Water flow rates were checked and adjusted daily to insure proper water exchange rate. Water temperature, salinity, dissolved oxygen, pH and ammonia were measured in nine randomly selected aquaria once per week. Ammonia was determined on a Technicon TA II AutoAnalyzer according to Bower and Bidwell (1978). Water temperature ranged from 25.0 to 27.0°C with an average of $25.1 \pm 0.4^\circ C$, salinity varied from 31.0 to 35.0 ppt with an average of 33.5 ± 0.9 ppt, and dissolved oxygen ranged from 5.3 to 7.8 mg/l with an average of 6.6 ± 0.6 mg/l. The pH values ranged from 6.1 to 7.9 with an average of 7.65 ± 0.2, and ammonia levels were between 0 and 0.042 ppm with an average of 0.0014 ± 0.003 ppm.

Every 14 days, the shrimp in each aquarium were counted and weighed. When the shrimp were removed for weighing, the aquaria were cleaned thoroughly and drained. On sampling days, shrimp were fed once in the afternoon with 60% of the amount of feed consumed the previous day to minimize cannibalism.

All diets were analyzed for dry matter, ash, crude protein and ether extract by AOAC methods (1980). Mineral analyses were done by inductive coupled atomic absorption spectrophotometer with a Perkin-Elmer 650. Water stability of pellets was determined at 2, 4, and 8 h, following the method of Lim and Destajo (1979).

Fifty shrimp were collected at the start of the experiment and stored at $-30^\circ C$ for determination of body composition. At the end of the experiment, all shrimp were collected and stored frozen at $-30^\circ C$ for subsequent chemical analyses. Moisture content of shrimp was determined by freeze drying the samples for 72 h. Proximate composition and mineral content of shrimp were determined following the same methods described above.

Feed conversion ratio was determined as the grams of air-dry weight of feed fed per gram of wet weight gain. The protein efficiency ratio was calculated as the grams of wet weight gain per gram of protein consumed. The apparent protein utilization was estimated as the grams of body protein increment per gram of protein consumed.

All data were subjected to analyses of variance and Tukey's test to determine the differences between the treatment means (Steele and Torrie, 1960). Results were considered statistically significant at the 0.05 probability level.

RESULTS

Average body weights are presented in Fig. 1. The growth response and feed utilization efficiency are shown in Table 2. Shrimp fed diets containing 0, 14

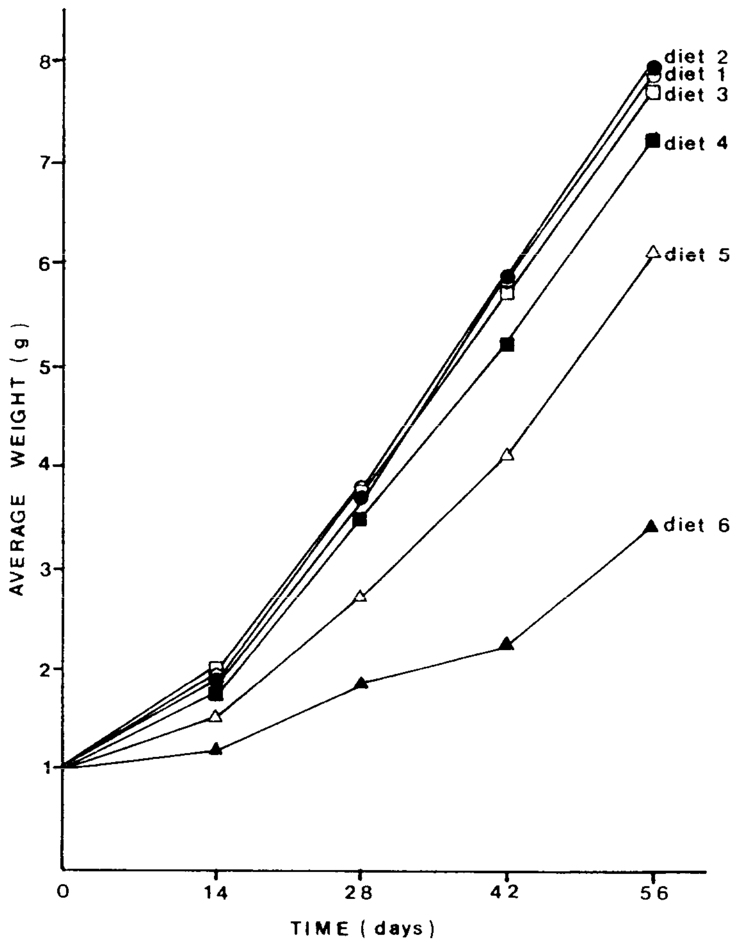

Fig. 1. Average weight of *P. vannamei* fed diets containing various levels of soybean meal.

TABLE 2

Growth response and feed utilization efficiency of shrimp fed diets containing various levels of soybean meal for 56 days[1]

Mean values	Diet number					
	1	2	3	4	5	6
Weight gain (g)	6.77 ± 0.36^{ab}	6.91 ± 0.40^{a}	6.56 ± 0.23^{ab}	6.15 ± 0.24^{b}	5.12 ± 0.14^{c}	2.34 ± 0.10^{d}
Survival (%)	90.33 ± 0.00^{bc}	90.00 ± 8.61^{cd}	96.67 ± 6.67^{ab}	98.33 ± 3.33^{a}	93.33 ± 5.44^{bc}	86.67 ± 0.00^{d}
Dry matter feed intake (g/shrimp)	10.94 ± 0.43^{a}	11.02 ± 1.02^{a}	10.22 ± 0.35^{a}	9.79 ± 0.62^{a}	8.43 ± 0.34^{b}	6.15 ± 0.45^{c}
Feed conversion	1.79 ± 0.07^{a}	1.79 ± 0.11^{a}	1.75 ± 0.12^{a}	1.77 ± 0.06^{a}	1.83 ± 0.08^{a}	2.87 ± 0.17^{b}
Protein efficiency ratio	1.48 ± 0.06^{a}	1.50 ± 0.09^{a}	1.53 ± 0.10^{a}	1.61 ± 0.06^{a}	1.48 ± 0.06^{a}	0.83 ± 0.04^{b}
Apparent protein utilization (%)	27.49 ± 1.12^{a}	27.29 ± 0.66^{a}	29.06 ± 1.38^{a}	28.44 ± 1.45^{a}	29.43 ± 0.46^{a}	16.08 ± 0.90^{b}
Protein gain (g/shrimp)	1.23 ± 0.06^{a}	1.23 ± 0.12^{a}	1.21 ± 0.04^{a}	1.16 ± 0.10^{a}	0.96 ± 0.03^{b}	0.39 ± 0.05^{c}
Fat gain (g/shrimp)	0.114 ± 0.016^{a}	0.108 ± 0.016^{ab}	0.106 ± 0.008^{ab}	0.095 ± 0.012^{ab}	0.088 ± 0.003^{b}	0.034 ± 0.007^{c}

[1]Values in the same row having the same superscript are not significantly different ($P > 0.05$).

and 28% soybean meal (diets 1, 2 and 3) exhibited similar growth patterns throughout the culture period. Growth rate declined when the level of soybean meal was 42% or greater (diets 4, 5 and 6). The mean weight gain of shrimp fed diet 2 was the highest and was significantly higher than those of shrimp fed diets 4, 5 and 6. No significant differences were found among the weight gains of shrimp receiving diets 1, 2 and 3. The weight gain of shrimp fed diet 4 was lower (although not significantly) than that of shrimp fed diets 1 and 3. The weight gains of shrimp on diets 1, 3 and 4 were statistically higher than those of shrimp fed diets 5 and 6. Shrimp receiving diet 6 had the lowest weight gain ($P<0.05$).

Shrimp fed diet 4 had significantly higher average survival than those of shrimp fed diets 1, 2, 5 and 6. Survival rates of shrimp fed diets 1, 3 and 5 did not differ significantly. Shrimp fed diet 6 had the lowest survival rate, although not significantly lower than that of shrimp on diet 2.

Total feed intake was nearly equal for diets 1 and 2 and was slightly higher than diets 3 and 4. Feed consumption significantly decreased when soybean meal levels were increased to 56 and 70% (diets 5 and 6). Shrimp fed diet 6 had the lowest feed intake.

The feed conversion ratios (FCR), protein efficiency ratio (PER) and the apparent protein utilization (APU) were essentially the same for diets 1 to 5. FCR, PER and APU of diet 6 were significantly lower than parameters measured for all other diets.

The protein gains were approximately the same for shrimp fed diets 1 to 4 and significantly higher than groups 5 and 6. Shrimp fed diet 6 had a significantly lower protein gain than shrimp fed the other diets.

Fat gains of shrimp tended to decrease with increasing levels of soybean meal in the diets. Shrimp fed the control diet (diet 1) had the highest fat gain; this was significantly higher than diets 5 and 6 but not different from diets 2, 3 and 4. Fat gain of shrimp fed diet 6 was the lowest ($P<0.05$). Values obtained for diets 2, 3, 4 and 5 did not differ significantly.

The average values of pellet water stability of various diets measured at 2, 4, and 8 h are shown in Fig. 2. The percentage of dry matter remaining were inversely related to the dietary level of soybean meal, but were nearly equal for diets containing the three lowest levels of soybean meal. Diet 6, which had the highest level of soybean meal, had the poorest water stability ($P<0.05$).

Whole body composition of shrimp expressed in percent dry matter, is presented in Table 3. Moisture content was highest for shrimp fed diet 6 ($P<0.05$); shrimp fed other diets were essentially the same. Percentages of crude fat, ash, calcium and potassium did not differ among the diets. Fat and ash content of shrimp generally decreased as the level of soybean meal in the diet increased. Shrimp fed diets 1 and 2 had similar and significantly lower body protein than shrimp fed diets 4 and 6. The protein content of shrimp fed diet 6 was the

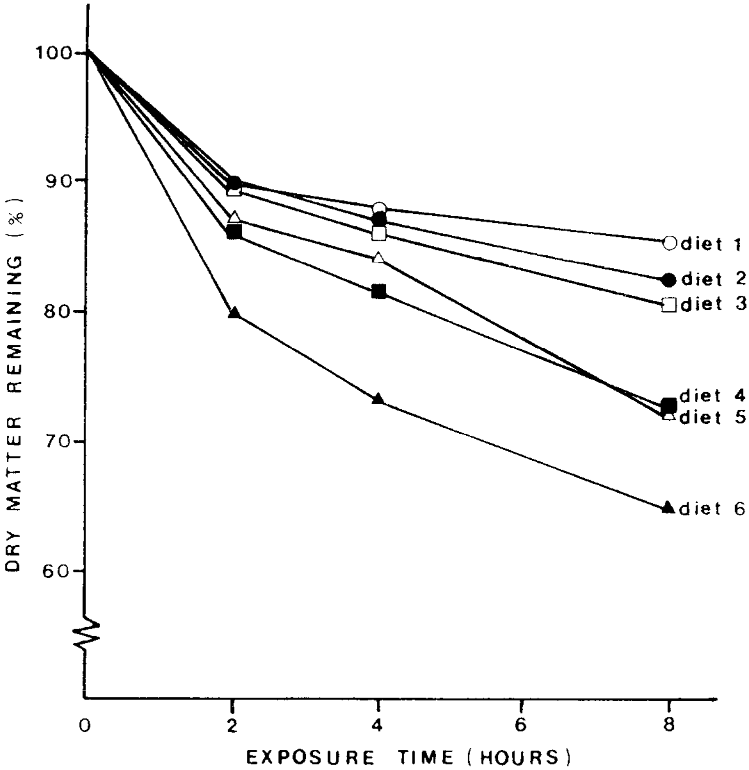

Fig. 2. Water stability of pellets containing various levels of soybean meal.

TABLE 3

Whole body percentage composition (dry matter) of shrimp fed diets containing various levels of soybean meal for 56 days[1]

Mean values	Diet number					
	1	2	3	4	5	6
Moisture	73.88±0.16[a]	73.96±0.29[a]	73.87±0.57[a]	73.91±0.42[a]	73.57±0.54[a]	76.46±1.25[b]
Crude protein	68.15±1.34[a]	68.15±0.75[a]	69.55±0.57[ab]	70.80±1.38[b]	70.00±1.70[ab]	71.10±1.30[b]
Crude fat	5.88±0.50	5.68±0.45	5.60±0.35	5.43±0.25	5.88±0.22	5.18±0.56
Ash	15.63±0.38	15.13±0.39	15.08±0.88	14.73±0.44	14.45±1.64	13.68±0.33
Calcium	2.70±0.12	2.90±0.29	3.15±0.31	2.97±0.33	3.10±0.56	2.88±0.15
Phosphorus	1.25±0.03[a]	1.24±0.03[ab]	1.27±0.04[a]	1.27±0.01[a]	1.18±0.02[bc]	1.12±0.02[c]
Potassium	1.38±0.05	1.28±0.10	1.38±0.10	1.35±0.06	1.35±0.10	1.33±0.05

[1]Values in the same row having the same superscript are not significantly different ($P > 0.05$).

highest, but not significantly different from those obtained with shrimp fed diets 3, 4 and 5. Phosphorus content of shrimp was essentially the same for shrimp fed diets 1, 2, 3 and 4. Shrimp fed diet 5 had significantly lower body phosphorus levels than those fed diets 1, 3 and 4. Shrimp fed diet 6 had the lowest body phosphorus content but not significantly lower than diet 5.

DISCUSSION

Weight gain of shrimp significantly decreased as levels of dietary soybean meal increased to 42% or higher. Survival rate was lowest for shrimp fed diet 6. However, this could not be attributed to the dietary level of soybean meal since no discernible trend among the values was obtained from various treatments. Protein and fat gains were closely correlated with weight gain and tended to decrease at soybean levels of 42% or greater.

Reduced gains in weight, protein and fat of shrimp fed diets with more than 28% soybean meal appeared to be related to palatability. The amount of feed consumed started to decline when the level of soybean meal in the diet exceeded 14%. Feed intake significantly decreased when the dietary level of soybean meal was higher than 42%. It has been reported that soybean products are unpalatable for some species of shrimp and fish. *P. aztecus* ingested significantly less feed when purified soybean protein was included in the diet at a level of 52% (Fenucci and Zein-Eldin, 1976). Chinook salmon refused to eat a diet containing 80% full-fat soybean heated at 230°C for 8 min (Fowler, 1980).

Pellet water stability was inversely related to the dietary level of soybean meal. The percentages of dry matter remaining decreased with increasing soybean meal level in diets. Pellet water stability tested at 4 and 8 h was significantly reduced when soybean meal level was 42% or higher. This may be attributed to the low binding properties of soybean meal. Snyder and Kwon (1987) indicated that soybean is rich in structural, high molecular weight carbohydrates, namely cellulose, hemicellulose and pectins, but usually contains less than 1% starch.

Based on growth, survival, gains in protein and fat, feed consumption and pellet water stability, a 28% dietary level of soybean meal as a replacement of 40% of the marine animal protein mix appears to be optimum for juvenile *P. vannamei*. However, feed conversion, protein efficiency ratio and apparent protein utilization were essentially the same for diets containing 0 to 56% soybean meal. This suggests that, if palatability and water stability of the pellet can be improved, the dietary level of soybean meal could be increased up to 56%. Colvin and Brand (1977) reported good growth and feed efficiency of *P. californiensis* when 42% soybean meal and 0.8% D,L-lysine were added to the diet as a replacement for 50% of a 1:1 mixture of menhaden fish meal and sun-dried shrimp meal. Fenucci et al. (1980) found that substitution of 6.3% squid meal with 6.2% soybean protein in diets for *P. setiferus* and *P. vannamei* resulted in good growth, survival and feed conversion. Lawrence et al. (1986)

indicated that soybean meal at a level of 20 to 50% of the diet could replace fish and shrimp head meals without affecting growth and survival of *P. schmitti*, *P. setiferus* and *P. vannamei*. With *P. monodon*, good growth and survival were obtained with a diet containing 40% soybean meal as a substitute for fish meal (Akiyama, 1989). However, Fenucci and Zein-Eldin (1976) obtained poor growth and feed efficiency of *P. aztecus* fed a diet containing 52% purified soybean protein.

When marine animal protein mix was totally replaced by soybean meal (diet 6), the growth response of shrimp and the nutritive value of the diet significantly decreased. Thus, when used as the sole protein source, soybean meal was poorly utilized by *P. vannamei*. Forster and Beard (1973) also observed a growth reduction of *Palaemon serratus* when all dietary fish meal was replaced by soybean meal. In contrast, Sick and Andrews (1973) reported that soybean meal provided better growth of *P. duorarum* than fish meal, shrimp meal, casein or corn gluten. Soybean cake particles have also been shown to be an effective feed for growth and survival of *P. japonicus* zoea (Hirata et al., 1975).

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